

# Comparative Morphology of the Sperm in Chondrichthyan Fishes

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## ABSTRACT

The external features of the spermatozoa of 35 species belonging to 19 families in 12 orders were observed with light and scanning electron microscopes. Six characters, i.e., type of sperm aggregate, number of helical gyres, total, head, midpiece and flagellum length, were used as an operational taxonomic unit for the computation of the resemblance of the sperm among species in a cluster analysis. The sperm of Orectolobiformes, Lamniformes, Carcharhiniformes, Torpediniformes and Rajiformes form sperm aggregates: spermatozeugmata and spermatophores. The sperm of 29 species are helical from the tip of the head to the midpiece, but *Chlamydoselachus anguineus*, *Dalatias licha*, *Etmopterus* spp., and *Squatina japonica* do not display a helical form. The sperm length ranges from 93  $\mu\text{m}$  to 224  $\mu\text{m}$ . The sperm of holocephalans have a shorter head and longer flagellum in proportion to that of elasmobranchs. The results of the cluster analysis suggest the external features of the sperm show a similarity within the genus and/or family.

## RÉSUMÉ

### Morphologie comparée des spermatozoïdes des poissons Chondrichtyens

La morphologie externe des spermatozoïdes de 35 espèces appartenant à 19 familles dans 12 ordres a été observée en microscopie photonique et à balayage. Six caractères, le type d'agrégation des spermatozoïdes, le nombre de tour de l'hélicoïde, la longueur du spermatozoïde, de la tête, de la pièce intermédiaire, du flagelle, ont été utilisés comme unités taxonomiques opérationnelles pour le calcul de la ressemblance des spermatozoïdes parmi les espèces par une méthode agglomérative. Les spermatozoïdes des Orectolobiformes, Lamniformes, Carcharhiniformes, Torpediniformes et Rajiformes forment des agrégations: spermatozeugmata et spermatophores. Les spermatozoïdes de 29 espèces sont hélicoïdaux à partir de l'extrémité de la tête jusqu'à la pièce intermédiaire, mais *Chlamydoselachus anguineus*, *Dalatias licha*, *Etmopterus* spp. et *Squatina japonica* n'ont pas de spermatozoïdes hélicoïdaux. La longueur des spermatozoïdes varie de 93  $\mu\text{m}$  à 224  $\mu\text{m}$ . Les spermatozoïdes des Holocephales ont une tête plus courte et un flagelle plus long que les Élasmobranches. Les résultats de l'analyse par méthode agglomérative suggèrent que la morphologie externe des spermatozoïdes est similaire à l'intérieur des genres et/ou des familles.

Chondrichthyans are a small group of about 900 species, compared with osteichthyans. They include two subclasses, 14 orders, and 51 families [1, 9]. Recently, the systematics and phylogeny of elasmobranchs have been considered using the external, skeletal and muscular systems of the body, and new schemes have been presented [2, 12]. Female chondrichthyan



fishes have diverse and complex reproductive styles, which have also been used as characters for consideration of their systematics and phylogeny [3, 10]. In contrast, the reproduction of male fishes is simple. Claspers, which are copulatory organs, differ in structure among species. Therefore, the claspers have traditionally been used to provide characters in taxonomy and systematics [5, 7].

During the last decade, electron microscopy (EM) has been refined. It provides new information on the fine structure and function of organs, tissues and cells in various animals. The sperm of many fishes has been observed with EM, and the interrelationships of fishes have been discussed in relation to the structure of the spermatozoa [6, 8]. However, comparative studies on the sperm of chondrichthyan fishes are few [4]. The present paper deals with the comparative morphology of the sperm in 35 chondrichthyan fishes on the basis of observations with light and scanning electron microscopes.

#### MATERIALS AND METHODS

Chondrichthyan fishes, belonging to 12 orders, 19 families and 35 species, were collected on the northeast coast of the United States, and in Suruga Bay and adjacent waters of central Japan from 1990 to 1994 (Table 1). Spermatozoa, seminal fluids and tissues were taken from various portions of the reproductive tract of the specimens just after the death. For observing under light microscopy, they were fixed in 10% neutral formalin. Sperm smears were prepared by smearing and air-drying seminal fluids on glass slides, and were used for measuring the dimensions of 30 sperm under Nomarski differential interference microscopy. For scanning electron microscopy (SEM), they were immersed in a cold fixative containing 2% paraformaldehyde and 2% glutaraldehyde in a 0.1 M cacodylate buffer (pH 7.3) with 10% sucrose added. They were rinsed in the above buffer, post fixed in 2% osmium tetroxide in the same buffer, dehydrated in graded ethanol, and displaced with *t*-butyl alcohol, frozen and dried in a vacuum desiccator. They were sputter-coated with gold, and observed and photographed using an Akashi ABT-55 scanning electron microscope.

For numerical taxonomy on the sperm of each species, six characters of the sperm: type of sperm aggregate, number of helical gyres, total length, head length, midpiece length, and flagellum length, were used as an operational taxonomic unit (OTU). The following five types of sperm aggregate were recognized: 1. a solitary sperm or sperm clump, 2. single-layered spermatozeugma, 3. compound spermatozeugma, 4. "rice-grain" type of spermatophore, 5. atypical rod shaped spermatophore [11]. The types were scored from 1 to 5, respectively. Data sets containing each character were standardized; values of each character were calculated as units of standard deviation from the mean value of each character. The correlation coefficient as a measure of the overall similarity was computed by comparison of each OTU pair. Clusters of the OTUs were created under the unweighted pair-group method using arithmetic averages (UPGMA).

#### RESULTS

##### *Observations with light and scanning electron microscopes.*

*Formation of sperm aggregates.* The sperm were separate within secretions in the epididymis of all species. In *Orectolobus japonicus*, 3 species of Lamniformes, 13 species of Carcharhiniformes, *Torpedo tokionis* and *Raja eglanteria*, the sperm in the ductus deferens formed clumps with heads adhering. They formed sperm aggregates in the ampulla ductus deferentis as reported in [11]. The type of sperm aggregate for each species is shown in Table 2. Single layered spermatozeugmata were found in 10 species of Carcharhiniformes except *Prionace glauca* and *Sphyrna lewini*, *T. tokionis*, and *R. eglanteria* (Fig. 1A). *O. japonicus* formed compound spermatozeugmata (Fig. 1B). The sperm aggregates of *P. glauca*, *S. lewini*, *Carcharias taurus*, and *Isurus oxyrinchus* have been described in detail [11]. *Alopias pelagicus* possessed rice-grain typed spermatophores. In *Heterodontus japonicus* and *Squatina japonica*, the sperm gathered in clumps in the ductus deferens as in the above species, and retained this arrangement in the ampulla ductus deferentis. Though *Chlamydoselachus anguineus*, 9 species of Squaliformes, *Rhinobatos schlegelii*, and 2 species of Myliobatiformes made sperm clumps temporarily, most of the sperm were solitary in the ampulla. The sperm of Chimaeriformes also formed clumps in the ductus deferens by adhesion of the midpieces, a condition differing from elasmobranchs. However, the sperm clumps were not found in the seminal fluid from the distal end of the ampulla.



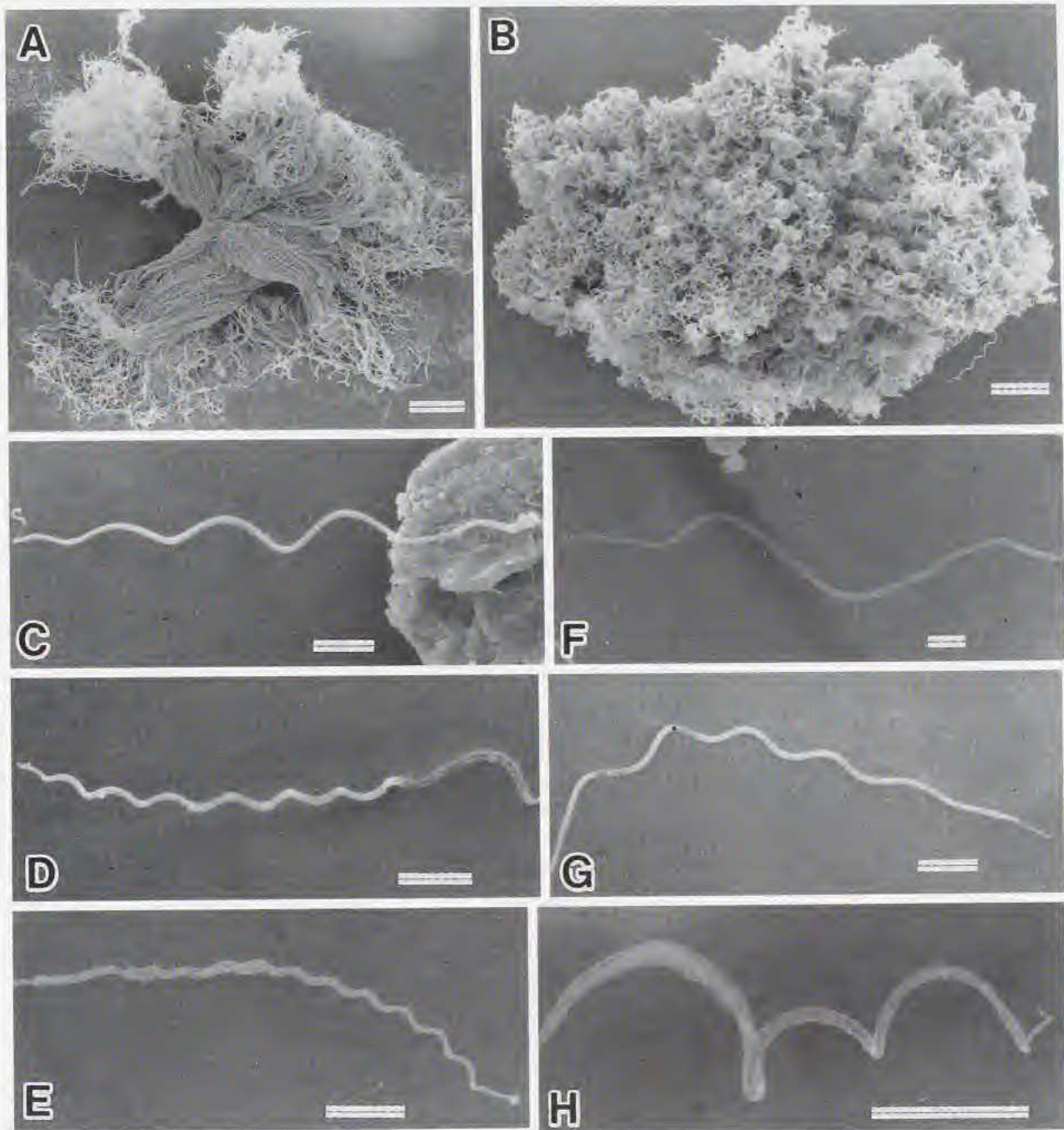


FIG. 1. — Sperm aggregate and the head and midpiece of sperm in several chondrichthyan fishes. **A:** Single layered spermatozeugma of *Galeus nipponensis*. **B:** Compound spermatozeugma of *Orectolobus japonicus*. **C:** Sperm of *Isurus oxyrinchus*. **D:** Sperm of *Mustelus griseus*. **E:** Sperm of *Sphyrna lewini*. **F:** Sperm of *Dalatias licha*. **G:** Sperm of *Rhinobatos schlegelii*. **H:** Sperm of *Chimaera phantasma*. Scale indicates 25  $\mu\text{m}$  in A and B, and 5  $\mu\text{m}$  in C to H.

TABLE 1. — Species and sample size of specimens from which sperm were obtained.

Heterodontiformes		Squaliformes	
Heterodontidae		Squalidae	
<i>Heterodontus japonicus</i>	1	<i>Centroscymnus owstoni</i>	3
Orectolobiformes		<i>Dalatias licha</i>	2
Orectolobidae		<i>Deania calcea</i>	2
<i>Orectolobus japonicus</i>	2	<i>Deania historicosa</i>	5
Lamniformes		<i>Etmopterus brachyurus</i>	3
Odontaspidae		<i>Etmopterus molleri</i>	1
<i>Carcharias taurus</i>	1	<i>Etmopterus pusillus</i>	2
Alopiidae		<i>Squalus brevirostris</i>	2
<i>Alopias pelagicus</i>	1	<i>Squalus japonicus</i>	3
Lamnidae		Squatiniiformes	
<i>Isurus oxyrinchus</i>	2	Squatinae	
Carcharhiniformes		<i>Squatina japonica</i>	1
Scyliorhinidae		Rhinobatiformes	
<i>Cephaloscyllium umbratile</i>	2	Rhinobatidae	
<i>Galeus eastmani</i>	5	<i>Rhinobatos schlegelii</i>	1
<i>Galeus nipponensis</i>	3	Torpediniformes	
Triakidae		Torpedinidae	
<i>Hemistriakis japonica</i>	4	<i>Torpedo tokionis</i>	2
<i>Mustelus canis</i>	1	Rajiformes	
<i>Mustelus griseus</i>	1	Rajidae	
<i>Mustelus manazo</i>	2	<i>Raja eglanteria</i>	2
Carcharhinidae		Myliobatiformes	
<i>Carcharhinus plumbeus</i>	1	Urolophidae	
<i>Galeocerdo cuvier</i>	1	<i>Urolophus aurantiacus</i>	2
<i>Prionace glauca</i>	3	Myliobatididae	
Sphyrnidae		<i>Myliobatis tobijei</i>	1
<i>Sphyrna lewini</i>	3	Chimaeriformes	
Hexanchiformes		Chimaeridae	
Chlamydoselachidae		<i>Chimaera phantasma</i>	1
<i>Chlamydoselachus anguineus</i>	1	<i>Hydrolagus mitsukurii</i>	2
		Rhinochimaeridae	
		<i>Rhinochimaera pacifica</i>	1

*External features of sperm.* The sperm of 35 species consisted of a head including an acrosome, a midpiece and a slender flagellum. The total length of the sperm ranged from 93  $\mu\text{m}$  in *Galeocerdo cuvier* to 224  $\mu\text{m}$  in *S. japonica* (Table 2). The external features of the head and midpiece of the sperm varied in each species (Fig. 1C-H). The head in most species was helical, but in *C. anguineus*, *Dalatias licha*, three species of the genus *Etmopterus*, and *S. japonica*, the sperm in the ampulla did not display a clear helical form in the head (Fig. 1F). The tip of the head was bent like a gaff (Fig. 1C, H). The number of gyres of the sperm with the helical form ranged from 3 to 24 (Table 2). The head length in elasmobranchs was more than 30  $\mu\text{m}$ , while that in holocephalans was less than 25  $\mu\text{m}$ . The longest sperm head was 93  $\mu\text{m}$  in *Centroscymnus owstoni*. The standard deviation of the head length in each species was 1.03 to 2.91  $\mu\text{m}$ . The proportion of the head to the total length ranged from 7 to 45 % (Table 2).

The midpiece was much shorter than the head in elasmobranchs. In contrast, two species of Chimaeridae had a long midpiece compared to the head. The width of the midpiece was slightly thicker than the head (Fig. 1C-H). The midpiece length ranged from 6 to 21  $\mu\text{m}$ , and the



TABLE 2. — Summary of the measurement and condition of the sperm in 35 chondrichthyan fishes.

Type of sperm aggregate: 1, Solitary or sperm clumps, 2, Single-layer spermatozeugmata, 3, Compound spermatozeugmata, 4, Spermatophores (rice-grain type), 5, Spermatophores (atypical rod). Values in parentheses indicate a ratio to total length.

Species	Code in Fig. 2	Type of sperm aggregate	Number of gyres	Total length in $\mu\text{m}$	Head length in $\mu\text{m}$ (ratio)	Midpiece length in $\mu\text{m}$ (ratio)	Flagellum length in $\mu\text{m}$ (ratio)
<i>Heterodontus japonicus</i>	HDJ	1	6	156	41 (26)	14 (9)	102 (65)
<i>Orectolobus japonicus</i>	OLJ	3	5	136	49 (36)	14 (10)	73 (54)
<i>Carcharias taurus</i>	CCT	5	17	110	37 (34)	13 (12)	60 (54)
<i>Alopias pelagicus</i>	APP	4	12	100	37 (37)	9 (9)	54 (54)
<i>Isurus oxyrinchus</i>	IRO	4	7	134	60 (45)	12 (9)	62 (46)
<i>Cephaloscyllium umbratile</i>	CSU	2	24	186	48 (26)	21 (12)	116 (62)
<i>Galeus eastmani</i>	GLE	2	13	197	45 (23)	19 (10)	133 (67)
<i>Galeus nipponensis</i>	GLN	2	14	197	44 (22)	19 (10)	134 (68)
<i>Hemitriakis japonica</i>	HTJ	2	23	120	46 (38)	14 (12)	60 (50)
<i>Mustelus canis</i>	MLC	2	11	98	41 (42)	7 (7)	50 (51)
<i>Mustelus griseus</i>	MLG	2	10	114	47 (41)	9 (8)	58 (51)
<i>Mustelus manazo</i>	MLM	2	12	122	38 (31)	10 (8)	74 (61)
<i>Carcharhinus plumbeus</i>	CRP	2	7	101	33 (33)	9 (9)	59 (58)
<i>Galeocerdo cuvier</i>	GCC	2	17	93	35 (38)	9 (10)	49 (52)
<i>Prionace glauca</i>	PNG	3	6	116	34 (30)	9 (7)	73 (63)
<i>Sphyrna lewini</i>	SRL	3	12	109	41 (38)	8 (7)	60 (55)
<i>Chlamydoselachus anguineus</i>	CLA	1	0	175	46 (26)	19 (11)	110 (63)
<i>Centroscyminus owstoni</i>	CTO	1	23	210	93 (44)	20 (10)	97 (46)
<i>Dalatias licha</i>	DTL	1	0	130	50 (38)	12 (10)	68 (52)
<i>Deania calcea</i>	DNC	1	3	141	56 (40)	13 (9)	72 (51)
<i>Deania historiosa</i>	DNH	1	3	162	55 (34)	15 (9)	92 (57)
<i>Etmopterus brachyurus</i>	EPB	1	0	193	63 (33)	16 (8)	114 (59)
<i>Etmopterus molleri</i>	EPM	1	0	178	58 (33)	16 (8)	104 (59)
<i>Etmopterus pusillus</i>	EPP	1	0	172	49 (28)	17 (10)	106 (62)
<i>Squalus brevirostris</i>	SLB	1	10	166	36 (22)	11 (7)	119 (71)
<i>Squalus japonicus</i>	SLJ	1	10	153	35 (23)	9 (6)	109 (71)
<i>Squatina japonica</i>	STJ	1	0	224	64 (29)	16 (7)	143 (64)
<i>Rhinobatos schlegelii</i>	RBS	1	6	131	46 (35)	9 (7)	76 (58)
<i>Torpedo tokionis</i>	TPT	2	5	130	51 (39)	10 (8)	69 (53)
<i>Raja eglanteria</i>	RJE	2	6	130	39 (30)	17 (13)	74 (57)
<i>Urolophus aurantiacus</i>	UPA	1	7	129	55 (44)	11 (8)	63 (48)
<i>Myliobatis tobijei</i>	MBT	1	5	96	35 (36)	10 (11)	51 (53)
<i>Chimaera phantasma</i>	CMP	1	3	137	11 (8)	11 (8)	115 (84)
<i>Hydrolagus mitsukurii</i>	HLM	1	3	164	11 (7)	18 (11)	135 (82)
<i>Rhinochimaera pacifica</i>	RCP	1	3	112	20 (18)	6 (5)	86 (77)

disparities among species were small. The standard deviation of the midpiece length in each species was 0.37 to 1.71  $\mu\text{m}$ . The flagellum length ranged from 49  $\mu\text{m}$  in *G. cuvier* to 143  $\mu\text{m}$  in *S. japonica*. The standard deviation of the flagellum length was 1.42 to 3.45  $\mu\text{m}$ . In *Isurus oxyrinchus*, *C. owstoni* and *Urolophus aurantiacus*, the flagellum was almost the same length as the head. The proportion of the flagellum in Chimaeriformes was more than 75%.

*Numerical taxonomic analysis.*

The cluster analysis indicated the similarity of external features of the sperm within the genus and/or family (Fig. 2). The sperm of the two species in *Galeus* and *Squalus* were similar, while the similarity in two species of *Deania* was low compared to the former genera. In *Etmopterus* and *Mustelus*, the sperm of two of the three species were much alike, but that of each remaining species was similar to that of other genera. The external features of the sperm in *Squalus* spp. were very different from those in the other genera of Squalidae. The similarity of the families, except Scyliorhinidae, within the Carcharhiniformes was almost equal to that of the genera, except *Squalus*, within the Squalidae. The cluster analysis also divided the 35 species into 4 groups (Fig. 2).

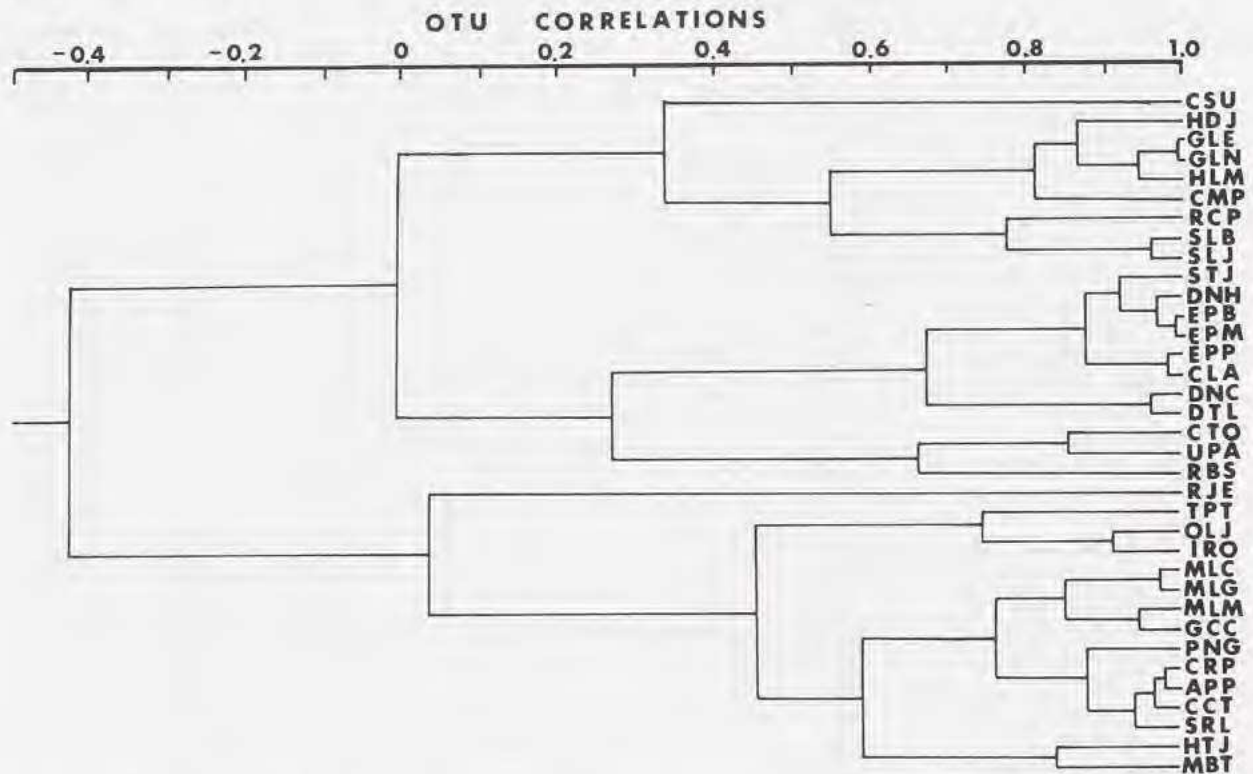


FIG. 2. — Phenogram of 35 species in chondrichthyan fishes, using six characters of sperm morphology in cluster analysis. Three letter codes at right indicate species name (see Table 2). Cophenetic correlation coefficient: 0.820.

*Group I CSU to SLJ.* This group included four orders, five families; Scyliorhinidae of the Carcharhiniformes, Heterodontiformes, two families of Chimaeriformes, and Squaliformes. Only the Scyliorhinidae was distant from the other families of Carcharhiniformes. The sperm of three species of Scyliorhinidae were much longer than those of the other species of Carcharhiniformes. *Cephaloscyllium umbratile*, in which the sperm had 24 gyres, differed from the other species in the group. The proportion of the head and flagellum in *Squalus* spp. was smaller and larger than that in the other genera of Squaliformes, respectively.

*Group II STJ to RBS.* This group consisted of five orders, five families; Squatiniformes, Squaliformes, Hexanchiformes, Urolophidae of Myliobatiformes, and Rhinobatiformes. Most of the species of Squalidae belonged to the group. Urolophidae was separated from Myliobatididae of the same order. The group was divided into two subgroups. The number of the gyres of the



sperm in the subgroup from STJ to DTL was less than that in the subgroup from CTO to RBS. The sperm of *C. owstoni*, alone in the Squalidae, had a large number of gyres.

*Group III RJE.* This group contained only one species, *Raja eglanteria*. The proportion of the midpiece in this species was large compared to the other species, while the sperm had a small number of gyres.

*Group IV TPT to MBT.* This group included five orders and nine families; Torpediniformes, Orectolobiformes, three families of Lamniformes, three families of Carcharhiniformes, and the Myliobatidae in the Myliobatiformes. All species of Carcharhiniformes except Scyliorhinidae belonged to this group. The group was divided into two subgroups. The sperm in the subgroup from TPT to IRO was longer than that in another subgroup. Only *I. oxyrinchus* was separated from the other species of Lamniformes. In the Triakidae, *Hemitriakis japonica* was separated from the other species. The sperm of *H. japonica* had a larger number of gyres than other species of the same family.

#### DISCUSSION

The internal morphology of the sperm, especially the structure of the flagellum, displays differences between elasmobranchs and holocephalans [4, 6, 8]. However, literature which deals with the relationship between the external features of the sperm and the phylogeny in chondrichthyan fishes is scanty. The external features of the sperm in chondrichthyan fishes were recognized to be species specific. They showed a similarity within the genus and/or family. Four groups based on the cluster analysis also suggested a similarity of sperm morphology within the order. COMPAGNO [1] and SHIRAI [12] divided elasmobranchs into four and two groups, respectively. Both authors recognized the four orders Heterodontiformes, Orectolobiformes, Lamniformes and Carcharhiniformes, as one group. The present study also recognized one group consisting of three orders excepting the Heterodontiformes. The remaining three groups of COMPAGNO: 1. Hexanchiformes, Squaliformes and Pristiophoriformes, 2. Batoids (Skates and Rays), and 3. Squatiniformes, are equal to the group 2 of SHIRAI. COMPAGNO [1] considered that the three groups are independently derived, while SHIRAI [12] regarded them as one of the two groups derived from a basal group. Group II in the present study includes five orders and is close to SHIRAI's grouping. COMPAGNO [2] divided Carcharhiniformes into two suborders; Scyliorhinoidei and Carcharhinoidei. The Scyliorhinidae of Group I belongs to the former, and the three families of Group IV belong to the latter.

COMPAGNO [2]: 4 groups	SHIRAI [12]: 2 groups
1. Heterodontiformes Orectolobiformes Lamniformes Carcharhiniformes	Group 1
2. Hexanchiformes Squaliformes Pristiophoriformes	Group 2
3. Batoids	
4. Squatiniformes	

The families of Group I, except two species of Squalidae, and only one family, Rajidae, of Group III are oviparous [3]. The formation of sperm aggregates has been demonstrated in various species of elasmobranchs [11]. In this study, it was found to be of the same type within the order. This may be related to the similarity of reproductive modes within the order [3]. Thus, the grouping of chondrichthyan fishes based on the external features of the sperm in this account reflects the systematics and phylogeny derived from consideration of the external, skeletal and muscular systems of the body and the female reproductive modes.

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